19. (Twice Amended) A retroviral vector according to claim 18 wherein the functional intron is positioned so that it restricts expression of at least one of the NOIs in a desired target site.

21. (Thrice Amended) A retroviral vector according to claim 1 wherein the vector or pro-vector is a murine oncoretrovirus or a lentivirus retroviral vector or pro-vector.

22. (Twice Amended) A retroviral vector according to claim 21 wherein the vector is a MMLV, MSV, MMTV, HIV-l, or EIAV retroviral vector.

23. (Thrice Amended) A retroviral vector as defined in claim 1 wherein the retroviral vector is an integrated provirus.

24. (Thrice Amended) A retroviral particle obtained from a retroviral vector according to claim 1.

42. (Thrice Amended) A retroviral vector according to claim 1 wherein said retroviral vector differentially expresses NOIs in target cells.

REMARKS

I. Introduction

Applicants respectfully request reconsideration and withdrawal of the rejections set forth in the Office Action.

Claims 20, 25-26, 28-29, 31-41, and 43-45 have been canceled, without prejudice or disclaimer thereof. Applicants reserve the right to prosecute the subject matter of these claims in this or another application.

In addition, claims 1-19, 21-24, and 42 have been amended. Details of the amendments are provided in the following discussion.

It is acknowledged that the amendments are made after final rejection of the application. However, because the foregoing amendments do not introduce new matter, and because the amendments place the application in condition for allowance, or at least in better condition for appeal, entry thereof by the Examiner is respectfully requested. Upon entry of this Amendment, claims 1-19, 21-24, 30, and 42 will be pending in the application.

II. Summary of the Invention

For the Examiner's convenience, Applicants include the following brief summary of their claimed invention.

The claimed invention provides a retroviral vector-based gene delivery system that efficiently expresses one or more nucleotides of interest (NOIs) at one or more desired target sites. The invention also provides a system for preparing high titers of retroviral vectors. This system incorporates safety features for efficient *in vivo* expression of one or more NOIs at one or more target sites.

The safety features of the retroviral vector include splice donor/acceptor sites that only become active upon transduction, *i.e.* following reverse transcriptase activity. In the parent vector (the RNA stage), the splice donor/acceptor sites are rendered inactive by placing the splice donor site downstream of the splice acceptor site. Translocation of the splice donor site to a location upstream of the splice acceptor site occurs via reverse transcription of the parent vector (the DNA stage). Transduction and insertion of the reverse transcribed DNA, which originates from the parent vector into the host cell genome, results in a pro-virus (DNA) that carries a functional intron containing a nucleic acid sequence of interest. However, because the gene of interest is bracketed by the intron, no transcription will take place from the gene due to splicing out of the sequence in mRNAs transcribed from the pro-virus. Thus expression of a second gene of interest that is positioned downstream of the splice acceptor site is activated because of the functioning intron (see page 25, lines 4-13, of the application).

The claimed invention provides highly desired *in vivo* stable gene expression, rather than transient gene expression. Specifically, the invention's retroviral vector, which is generated from primary target cells, can transduce secondary target cells. Furthermore, integration of the retroviral genome into the host's DNA ensures stable maintenance of gene expression in the secondary target cells. These secondary target cells do not express significant amounts of viral antigens and are therefore less immunogenic than cells transduced with adenoviral vector (see page 45, lines 5-12 of the application).

Moreover, use of the claimed invention's retroviral vector as a secondary vector enables targeting of specific cell types, such as rapidly dividing cells, as well as limited gene expression to a

primary or secondary target site. This eliminates a NOI's possible toxicity or antigenicity (see page 45, lines 20-25, of the application).

III. Rejections under 35 U.S.C. § 112, 2nd Paragraph

Claims 1-26, 28, 30, and 42 were rejected under 35 U.S.C. § 112, 2nd paragraph, as allegedly not providing an enabling disclosure for any nucleotide of interest, as claimed. The Examiner did acknowledge, however, that the application enables the selective expression of the hygromycin-neomycin gene pair or the hygromycin-p450 gene pair.

A. Skilled Artisans Can Detect and Manage Cryptic Splice Donor/Acceptor Sites

As one basis for the enablement rejection, the Examiner asserted that the selective expression of foreign genes is unpredictable. In particular, the Examiner noted that cryptic splice donor/acceptor sites pose a potential challenge. Applicants respectfully traverse this ground for rejection.

Prior to the claimed invention, skilled artisans were aware of cryptic splice donor/acceptor sites, could readily detect them, and could readily eliminate them. Applicants enclose herewith copies of two articles evincing that cryptic splice/donor sites were known in the art: Sebillon et al., *NAR*, 1995, 23:3419025 and Maruyama et al., *Eur. J. Biochem.*, 1995, 232:700-05. Means for identifying such sites included performing sequence analysis, especially in those cases where transcripts of unexpected lengths were obtained. Prior to the claimed invention, those skilled in the art also knew that mutating cryptic splice sites leads to improved levels of gene expression. In 1996, Reichel *et al.* reported that removal of a cryptic splice site significantly improve GFP performance. Reichel *et al.*, *PNAS*, 1996, 93:5888-93. Similarly, Burn *et al.* reported in 1995 that the modification of a plasmid sequence to eliminate a cryptic splice event optimized the plasmid's performance. Burn *et al.*, *Gene*, 1995, 161:183-87. Applicants enclose herewith copies of the Reichel and Burn references for the Examiner's review.

Because those skilled in the art at the time of filing could readily detect and manage cryptic splice/donor sites, the application is enabling for more than just the working examples. Applicants therefore respectfully request withdrawal of this rejection.

B. Gene Therapy

As a second basis for the enablement rejection, the Examiner asserted that gene therapy is an unpredictable art. For the sole purpose of advancing the prosecution of this application,

Applicants have canceled all claims directed to gene therapy. Therefore, the rejection is moot. However, Applicants reserve the right to pursue the subject matter of these claims in this or another application.

IV. Rejections under 35 U.S.C. § 112, 1st Paragraph

Claims 1-26, 28, 30, and 42 were rejected under 35 U.S.C. § 112, 1st paragraph, as allegedly being indefinite. The Examiner asserted that in claim 1, it is unclear how the "retroviral vector" correlates to the "retroviral pro-vector." The Examiner also asserted that nucleic acid sequences do not "encode" splice sites and that it is unclear how limitations describing the retroviral pro-virus limit the retroviral vector of claim 1.

Applicants have amended claim 1 to clarify the relationship between the retroviral vector and the retroviral pro-viral vector: the retroviral vector is formed as a result of reverse transcription of a retroviral pro-vector. Given this relationship, it is apparent that limitations of the retroviral pro-vector will affect the retroviral vector. Applicants have also amended the claims to recite that the nucleic acid sequences contain, rather than encode, splice sites. None of these amendments change the scope of the claims.

Because the amended claims are definite, Applicants respectfully request that the Examiner withdraw the rejection.

V. Rejections under 35 U.S.C. § 102

Claims 1-6, 9-25, and 43-44 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by three references: (1) Morgenstern *et al.*, Nucleic Acids Research, Vol. 18, No. 12, 3587 (1990); (2) Takeda *et al.*, Nature, Vol. 314, April 4, 1985, pp. 452-54; and (3) Kriegler *et al.*, Cell, Vol. 38, September 1984, pp. 483-491. Applicants respectfully traverse the rejections.

A. Morgenstern *et al.* Do Not Describe the Claimed Invention

Claims 1-6, 9, 10, 12-15, 18-25, and 43-44 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Morgenstern *et al.* According to the Examiner, Morgenstern *et al.* describe a retroviral vector comprising a functional splice donor site and a functional splice acceptor site, including the limitations of each rejected claim.

Contrary to the Examiner's assertion, Morgenstern *et al.* do not describe the claimed invention. Although Morgenstern *et al.* provide some vectors with functional splice donor sites (*e.g.*, pRLV S+), the splice donor sites are not positioned according to the recitations of claim 1. The specific positioning in the claimed retrovirus vectors represents an important safety feature that Morgenstern *et al.* lacks: it ensures that the retroviral vector's splice donor/acceptor sites only become active upon transduction (*see, e.g.*, specification, pg. 25, ln. 9-13). This directly contrasts with the vectors of Morgenstern *et al.*, which employ direct orientation (DO) retroviral vectors.

Specifically, the retroviral vector of the claimed invention derives from a retroviral pro-vector that comprises a first nucleotide sequence (NS) capable of yielding a functional splice donor site and a second NS capable of yielding the functional splice acceptor site, wherein the first NS is positioned downstream of the second NS such that the retroviral vector is formed *only* as a result of reverse transcription of the retroviral pro-vector. Reverse transcription of the pro-vector reshuffles the first and second NSs and arranges them in the correct, functional order. This rearrangement is a necessary prerequisite for retroviral vector formation before integration. Thus, there are both structural and functional relationships between the pro-vector and the integrated form of the retroviral vector of the claimed invention that Morgenstern *et al.* lacks.

The relative positioning set forth in claim 1 enables the vectors of the claimed invention to be used for splicing when the vector is transduced into a host cell genome (which can be advantageous, *e.g.*, in allowing splicing out of a packaging signal, allowing increased expression, *etc.*), but avoids undesired splicing when the vector is in virion RNA form (thereby avoiding the problem of low titer). The feature also significantly improves the *in vivo* utility of the retroviral vector.

This is in complete contrast to the general teachings of Morgenstern *et al.*, which provide an efficient "splice deficient" vector (pg. 3588, col. 2, ¶ 1). Furthermore, neither type of vector generated by Morgenstern *et al.*(those containing "splice deficient" nor those containing "splice functional" sites), requires reverse transcriptase activity to functionally align the splice donor and splice acceptor sites for achieving effective splicing.

Because Morgenstern *et al.* do not describe the claimed invention, Applicants respectfully request that the Examiner withdraw the rejection.

B. Takeda *et al.* Do Not Describe the Claimed Invention

Claims 1 and 15-17 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Takeda *et al.* According to the Examiner, Takeda *et al.* teach a retroviral vector that comprises a nucleic acid sequence encoding immunoglobulin genes and several splice donor/acceptor pairs.

In contrast to the Examiner's assertions, Takeda *et al.* do not teach the claimed invention. Specifically, Takeda *et al.* do not provide any regions encoding splice donor sites within a long terminal repeat (LTR) of the retrovirus. This is clear from Figure 1 of the reference, which shows that the insertion of a heterologous sequence did not disrupt the LTRs. Instead, the heterologous sequence was inserted between LTRs. Thus, Takeda *et al.* took a different approach from that of the claimed invention,, which did not provide the previously discussed advantages and safety features of the claimed invention.

Because Takeda *et al.* do not describe the claimed invention, Applicants respectfully request that the Examiner withdraw the rejection.

C. Kriegler *et al.* Do Not Describe the Claimed Invention

Claims 1 and 9-11 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Kriegler *et al.* According to the Examiner, Kriegler *et al.* teach retroviral vectors containing several splice donors/acceptors and the early genes of SV40, which includes the small t antigen.

In contrast to the Examiner's assertions, Kriegler et al. do not teach the claimed invention.

As with Takeda *et al.*, Kriegler *et al.* do not provide any regions encoding splice donor sites within a long terminal repeat (LTR) of the retrovirus. This is clear from Figure 1(d) of the reference, which shows that the insertion of a heterologous sequence did not disrupt the LTRs. Instead, the heterologous sequence was inserted between LTRs. Thus, Kriegler *et al.* took a different approach from that of the claimed invention, which did not provide the previously discussed advantages and safety features of the claimed invention.

Because Kriegler et al. do not teach the claimed invention, Applicants respectfully request that the Examiner withdraw the rejection.

VI. Conclusion

The present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

If there are any fees due in connection with the filing of this Amendment, please charge the fees to our Deposit Account No. 19-0741. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Date: January 2, 2002

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Marked Up Version of Claims with Markings to Show Changes

1. (Twice Amended) A retroviral vector [delivery system] comprising:

(a) a first nucleotide sequence ("NS") containing [encoding] a functional splice donor site; and

(b) a second NS containing [encoding] a functional splice acceptor site; wherein:

(i) the functional splice donor site and the functional splice acceptor site flank a first nucleotide sequence of interest ("NOI");

(ii) the functional splice donor site is upstream of the functional splice acceptor site; and

(iii) the retroviral vector is formed as a result of reverse transcription of a retroviral pro-vector, wherein the retroviral pro-vector comprises:

(a) a first nucleotide sequence ("NS") containing [encoding] the splice donor site; and

(b) a second NS containing [encoding] the splice acceptor site; wherein the first NS is downstream of the second NS; such that the retroviral vector comprising a first NS containing [encoding] a functional splice donor site and a second NS containing [encoding] a functional splice acceptor site is formed as a result of reverse transcription of the retroviral pro-vector.

2. (Twice Amended) A retroviral vector [delivery system] according to claim 1 wherein the retroviral pro-vector comprises a third NS that is upstream of the second NS; wherein the third NS contains [encodes] a non-functional splice donor site in the retroviral vector.

3. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the retroviral vector further comprises a second NOI; wherein the second NOI is downstream of the functional splice acceptor site.

4. (Thrice Amended) A retroviral vector [delivery system] according to claim 3 wherein the retroviral pro-vector comprises the second NOI; wherein the second NOI is upstream of the second NS.

5. (Thrice Amended) A retroviral vector [delivery system] according to claim 3 wherein the second NOI, or the expression product thereof, is or comprises a therapeutic agent or a diagnostic agent.

6. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the first NOI, or the expression product thereof, is or comprises any one or more of an agent conferring selectability, a viral essential element, or a part thereof, or combinations thereof.

7. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the first NS is at or near to the 3' end of a retroviral pro-vector.

8. (Thrice Amended) A retroviral vector [delivery system] according to claim 7 wherein the first NS of the retroviral pro-vector comprises a third NOI; wherein the third NOI is any one or more of a transcriptional control element, a coding sequence, or a part thereof.

9. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the first NS is a viral NS.

10. (Twice Amended) A retroviral vector [delivery system] according to claim 9 wherein the first NS is an intron or a part thereof.

11. (Twice Amended) A retroviral vector [delivery system] according to claim 10 wherein the intron is the small t-intron of SV40 virus.

12. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the retroviral pro-vector comprises a retroviral packaging signal; and wherein the second NS is located downstream of the retroviral packaging signal such that splicing is prevented at a primary target site.

13. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the second NS is placed downstream of the first NOI such that the first NOI is expressed at a primary target site.

14. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the second NS is placed upstream of a multiple cloning site such that one or more additional NOIs may be inserted.

15. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the second NS is a nucleotide sequence coding for an immunological molecule or a part thereof.

16. (Twice Amended) A retroviral vector [delivery system] according to claim 15 wherein the immunological molecule is an immunoglobulin.

17. (Twice Amended) A retroviral vector [delivery system] according to claim 16 wherein the second NS is a nucleotide sequence coding for an immunoglobulin heavy chain variable region.

18. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the vector additionally comprises a functional intron.

19. (Twice Amended) A retroviral vector [delivery system] according to claim 18 wherein the functional intron is positioned so that it restricts expression of at least one of the NOIs in a desired target site.

21. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein the vector or pro-vector is a murine oncoretrovirus or a lentivirus retroviral vector or pro-vector.

22. (Twice Amended) A retroviral vector [delivery system] according to claim 21 wherein the vector is a MMLV, MSV, MMTV, HIV-l, or EIAV retroviral vector.

23. (Thrice Amended) A retroviral vector [delivery system] as defined in claim 1 wherein the retroviral vector is an integrated provirus.

24. (Thrice Amended) A retroviral particle obtained from a retroviral vector [delivery system] according to claim 1.

42. (Thrice Amended) A retroviral vector [delivery system] according to claim 1 wherein said retroviral vector [delivery system] differentially expresses NOIs in target cells.